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The Effect of Giving Human Umbilical Cord Mesenchymal Stem Cells (HUMSC) on the Expression of Collagen I in Menopause Model Mice

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ABSTRACT

Background: Patients with pelvic floor dysfunction (PFD) who fail conservative treatment will undergo surgery. In recent years, various therapeutic approaches for PFD using stem cells have been investigated in vivo. One component of stem cells is the human umbilical cord mesenchymal stem cell (HUMSC). It is hoped that HUMSC can modify the collagen and elastin content in the extracellular matrix, leading to improvements in pelvic floor strength and PFD. **Methods:** This research is an experimental study with a randomized posttest-only controlled group design with the aim of determining the effect of giving HUMSC on the expression of Collagen I in a female mouse model of menopause. There are inclusion criteria, namely female Balb/c mice, healthy, never mated, aged 8 - 12 weeks, and body weight 18 - 22 grams. The research will be carried out with statistical analysis using SPSS; a p-value <0.05 is considered significant. **Results:** The results of the analysis of the distribution of sample means show that there are significant differences between the two groups. The treatment group had a higher mean expression of type I collagen compared to the control group. In addition, variation in the control group was lower compared with the treatment group, which could be interpreted as higher consistency in the response to HUMSC treatment. **Conclusion:** Human umbilical cord (HUMSC) has a positive impact on type I collagen expression in menopausal model mice.

1. Introduction

Currently, treatment for pelvic floor dysfunction (PFD) is symptom-based only. Women who have failed conservative treatment will undergo surgery. Autologous tissue or fascial repair always shows a high recurrence rate of approximately 11%, as reported by Weintraub et al.¹ Persistent pain and erosion of the urethra and/or bladder are the most common complications following transvaginal mesh (TVM) based repair. Other complications include infection and urinary retention. According to the FDA, more than 1000 adverse events related to complications after mesh implantation have been reported with POP and IUS treatment during the period 2005-2007, while the number of adverse events

continued to increase between 2008 and 2010. Thus, POP repair using TVM has not been shown to increase clinical benefit, with almost 30% of patients requiring subsequent surgery.²

In recent years, various therapeutic approaches for PFD using human umbilical cord mesenchymal stem cells (HUMSC) have been investigated in vivo. The application of HUMSC in the repair of pelvic tissue disorders has attracted more and more researchers.³ HUMSC has the self-renewal capacity, multilineage differentiation potential, growth factor secretion, and immunomodulation. Under specific induction, HUMSC differentiates into various types of pelvic floor supporting tissue cells, including muscle cells and fibroblasts, to compensate for damaged tissue cells

and facilitate tissue repair. In addition, HUMSC also has anti-apoptotic, anti-inflammatory, and provascular formation abilities.⁴

One component of stem cells is the human umbilical cord mesenchymal stem cell (HUMSC), which is a potential cell source and has various advantages, such as being easy to obtain in large quantities, cultured, and then expanded *in vivo*. Based on the histological findings, HUMSC transplantation resulted in the formation of well-organized connective tissue. The collagen type I/III ratio increased, indicating an adequate healing process, making HUMSC have a potential role in the problem of pelvic organ prolapse.⁵ The study aims to observe the effect of administering HUMSC on collagen I expression in menopausal model mice.

2. Methods

This research is an experimental study with a randomized posttest-only controlled group design with the aim of determining the effect of giving HUMSC on the expression of collagen I in a female mouse model of menopause. There are inclusion criteria, namely female Balb/c mice, healthy, never mated, aged 8 - 12 weeks, and body weight 18 - 22 grams. Based on Federer's formula, the sample size per group in this study was 18. The sample was divided into 2 groups, namely the control group (C), namely mice that were not given HUMSC, and the treatment group (T), namely mice that were given HUMSC.

Two weeks after ovariectomy, mice received a subepithelial injection of 0.3 ml saline: 3×10^6 HUMSC in the anterior vaginal wall. Before implantation, P3-5 HUMSC were labeled with a DiR fluorescent reagent (XenoLight). According to the manufacturer's instructions, 1×10^6 HUMSC were incubated with 1 ml of 80 g/ml DiR for 5 min at 37°C. Then, cells were rinsed with PBS twice. At 12 weeks after injection for each group (n=18/group for each group at 12 weeks), animals were sacrificed, and the anterior vaginal wall was harvested. After euthanasia, the abdominal cavity is dissected, and disarticulate the pubic symphysis. First, the vaginal canal is isolated from the

surrounding perineal skin. Then, the vaginal canal is isolated intact. The anterior vaginal wall was collected for testing and sectioned transversely into proximal (approximately upper two-thirds) and distal (approximately lower third) segments (n=6/group/time point). The proximal segment is opened longitudinally into two parts. Half were immediately immersed in 10% neutral buffered formalin, processed, and embedded in paraffin blocks to be used for histology and immunohistochemistry, and the other half were embedded in optimal cutting temperature (OCT) compound and stored at -80°C until cryosectioning. Distal segments were frozen in liquid nitrogen and stored at -80°C for assessment of measured mRNA expression. The anterior vaginal wall was collected and wrapped in wet gauze soaked in 0.9% normal saline. Tissues were stored at 0-4°C, and biomechanical testing was performed within 4 h of tissue harvest.

Then, the independent variable of this research is human umbilical mesenchymal stem cells. The dependent variable is collagen expression, and the control variables are age, body weight, environment, diet, and random variables in the form of genetics. The research will be carried out with statistical analysis using SPSS; a p-value <0.05 is considered significant.

3. Results

In the initial stage of analysis, a normality test was carried out using the Kolmogorov-Smirnov test and the Shapiro-Wilk test for the two variables, namely the treatment group and the control group. The test results showed that type I collagen expression data did not follow a normal distribution. The Kolmogorov-Smirnov test produced a statistical value of 0.293 (df = 19, Sig. = 0.000) for the treatment group and 0.275 (df = 19, Sig. = 0.001) for the control group. The results of the Shapiro-Wilk test also consistently indicate non-normality of data distribution with a statistical value of 0.773 (df = 19, Sig. = 0.0001) for the treatment group and 0.780 (df = 19, Sig. = 0.001) for the control group (Table 1).

Table 1. Normality test using the Shapiro-Wilk test.

| Group | statistic | df | p-value |
|-----------|-----------|----|---------|
| Control | 0.780 | 19 | 0.001 |
| Treatment | 0.773 | 19 | 0.0001 |

Normality test results that do not meet the normal distribution assumption are an important indication in selecting an appropriate analysis method. Although the data did not meet the assumption of a normal distribution, we chose to use the Kruskal-Wallis test as the next analysis method because this test does not depend on the assumption of a normal distribution.

In the context of non-normality of data distribution, the Kruskal-Wallis test was carried out to evaluate the difference in mean expression of type I collagen between the treatment group and the control group. The results of the Kruskal-Wallis test produced significant findings, with a p-value of 0.0001 (table 2). These results imply a significant difference in mean

type I collagen expression between the two groups.

These results strengthen the initial hypothesis that the administration of human umbilical cord mesenchymal stem cell (HUMSC) has a significant effect on the expression of collagen type I in menopausal model mice. Although the data were not normally distributed, the Kruskal-Wallis test provided strong support for the positive effect of HUMSC therapy on type I collagen expression. The researcher carried out an analysis of the distribution of sample means to provide a deeper understanding of the characteristics of the data in the two groups. Descriptive statistics for each group are shown in Table 2.

Table 2. Analysis of the distribution of sample means.

| Group | Means±SD | Median (min-max) | p-value |
|-----------|-------------|------------------|---------|
| Control | 1.168±0.889 | 1.1(0.2-4.1) | 0.0001 |
| Treatment | 2.921±0.971 | 3.2(0.9-3.9) | |

The results of the analysis of the distribution of sample means show that there are significant differences between the two groups. The treatment group had a higher mean expression of type I collagen compared to the control group. In addition, variation in the control group was lower compared with the treatment group, which could be interpreted as higher consistency in the response to HUMSC treatment.

4. Discussion

The results of this research reveal very interesting findings and have very important implications. These findings indicate that administration of human umbilical cord mesenchymal stem cells (HUMSC) has a significant effect on the expression of type I collagen in the anterior vaginal wall of menopausal model mice. Although the data obtained does not follow a normal

distribution, the results of non-parametric tests with the Kruskal-Wallis test provide strong evidence that supports significant differences between the two groups, namely the treatment group and the control group. Similar findings were also obtained by studies that performed HUMSC transplantation as a potential strategy to treat urinary and fecal incontinence. Mice given intravenous or urethral administration of HUMSC showed an increase in smooth muscle cells and elastin. Apart from that, an increase in vascular density and connective tissue in the periurethra was also found.⁵

These findings have a direct impact on the health of women who experience pelvic floor dysfunction (PFD) during menopause. PFD is a health problem that can disrupt women's quality of life and well-being and often requires invasive surgical procedures for effective

treatment. These findings open the door to more innovative and non-invasive treatment approaches. Giving stem cells can cause a rejection reaction by the body, which leads to a decrease in the effectiveness of therapy. However, HUMSC has the ability to modulate the immune system and provide anti-inflammatory effects, thus suppressing the body's excessive rejection response to foreign objects.⁶ Another study conducted by Mao et al. showed that HUMSC normalized the fibromuscular structures of the ovariectomized rat vagina after direct injection into the anterior vaginal wall.⁷

These results consistently support the initial hypothesis that HUMSC has the potential to increase the expression of type I collagen. Type I collagen is an important component in connective tissue, and increasing its expression can have a positive impact on connective tissue integrity. The integrity of this connective tissue is closely related to bone and skin health, which is often the main problem faced by individuals during menopause. The significant difference in mean type I collagen expression between the two groups is an interesting and important finding. This can be interpreted as a positive impact of HUMSC therapy in improving the quality of connective tissue in menopausal model mice. Healthier connective tissue can help reduce the risk of bone disorders such as osteoporosis and can also have a positive impact on skin health.⁸

The lower variation in the control group reflects consistency in response to therapy. This indicates that the control group had a more stable response to factors influencing type I collagen expression. On the other hand, the treatment group showed greater variation in response to HUMSC treatment. This could mean that there are varying individual responses to HUMSC therapy, which may be influenced by factors that have not yet been identified. The mechanism of action through in vivo research shows that exosomes increase type I collagen by increasing collagen synthesis and reducing collagen degradation in vaginal fibroblasts.⁶ Other mechanisms in animal models for HUMSC, such as improved reproductive

senescence through paracrine, anti-apoptotic, anti-fibrotic, angiogenic, anti-inflammatory immunomodulatory, and anti-oxidative stress effects, also perform well in restoring ovarian morphology and improving ovarian reserve capacity.⁹

The results of this study provide a strong basis for further research in an effort to understand the mechanisms underlying the positive effects of HUMSC on type I collagen expression in the anterior vaginal wall. Additionally, these findings may pave the way for the potential development of more effective therapies in treating health problems associated with PFD during menopause, which is a significant problem for many individuals, especially women. Moving forward, further research and clinical trials may be needed to support the use of HUMSCs in a broader therapeutic context. With this innovative approach, it is hoped that we can provide a safer and more effective solution for women who need treatment for PFD during menopause.

5. Conclusion

Human umbilical cord (HUMSCs) has a positive impact on type I collagen expression in menopausal model mice.

6. References

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